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A chip-based capillary electrophoresis-contactless conductivity microsystem for fast measurements of low-explosive ionic components

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A miniaturized analytical system for separating and detecting inorganic explosive residues, based on the coupling of a micromachined capillary electrophoresis (CE) chip with a contactless conductivity detector is described. The low electroosmotic flow (EOF) of the poly(methylmethacrylate) (PMMA) chip material facilitates the rapid switching between analyses of cations and anions using the same microchannel and run buffer (and without an EOF modifier), and hence offers rapid (< 1 min) measurement of seven explosive-related cations and anions. Experimental parameters relevant to the separation and detection processes have been optimized. Addition of a 18-crown-6 ether modifier has been used for separating the peaks of co-migrating potassium and ammonium ions. The ionic-explosive microchip system combines the distinct advantages of contactless conductivity detection with the attractive features of plastic CE microchips. The new microsystem offers great promise for monitoring explosive-related ions at the sample source, with significant advantages of speed/warning, efficiency, cost, or sample size.

Introduction

Growing concerns regarding terrorist bombings have generated tremendous demands for innovative tools capable of detecting major explosives in a faster, simpler, and reliable manner at the site of terrorism. Commercial and improvised explosives comprised of both inorganic and organic components have been used in related terrorist activity. The determination of specific ions, such as ammonium, methylammonium, potassium, sodium, perchlorate, chloride or nitrate is often crucial for the proper identification of explosives and their residues at terrorist or crime scenes.[1–4] The ionic components of the post-blast residue often reflect the original composition of the corresponding explosive device.[2] Such inorganic explosives and explosive residues are commonly analyzed using laboratory-based ion-chromatography or capillary-electrophoresis systems.[2–5] For example, McCord *et al.*[2] demonstrated the forensic analysis of bomb residue by capillary electrophoresis (CE) separating eight low-explosive components. Unfortunately, these laboratory-based methods are not field portable techniques, nor can they analyze relevant samples within extremely short (< 1 min) time scales. Accordingly, fast-responding field-deployable systems are urgently desired for monitoring ionic explosives at the sample source (before, during, or after a terrorist attack). Such portable devices should offer significant advantages in terms of speed/warning, efficiency, cost, or sample size. In particular, rapid identification of explosive-related ions will allow first responders to make the important decisions concerning evacuating, barricading, or efficient decontamination of a particular site, and will prevent them from becoming victims themselves.

This paper describes a chip-based CE-contactless conductivity microsystem for the rapid separation and detection of explosive-related ions. Lab-on-a-chip technology offers tremendous potential for obtaining the desired forensic information in a faster, simpler, and cheaper manner compared to traditional laboratory-based instruments. Particularly attractive for on-site counter-terrorism applications is the very small footprint of microfluidic devices, their very fast response, versatility, and high performance. Such attractive properties have led to the development of microchip CE devices for monitoring organic (nitroaromatic) explosives,[6–8] but not for ionic explosives. The present ionic-explosive microchip system combines the distinct advantages of contactless conductivity detection, such as effective isolation from high separation voltages and simplified construction,[9–14] with the attractive features and disposability of plastic CE microchips.[15,16] The low electroosmotic flow (EOF) of the PMMA chip material facilitates the rapid switching between analyses of cations and anions using the same microchannel and run buffer (without an EOF modifier), and hence offers rapid (< 1 min) measurement of seven explosive-related cations and anions. The optimization and performance characteristics of the new ionic-explosive microchip system are reported in the following sections.

Experimental

Apparatus

The PMMA microchips were manufactured at the Institut für Mikrotechnik Mainz (IMM, Mainz, Germany) and were described earlier.[16] The microchips consisted of two sealed PMMA plates (70 × 24 mm), with thickness of 1 mm (bottom) and 125 μm (top). The chip had a 60 mm long separation channel (between the run buffer reservoir and the outlet reservoir) and 18 mm long injection channel (between the sample reservoir and the unused reservoir) (Fig. 1E). The two channels crossed each other halfway between the sample and the unused reservoir and 9 mm from the run buffer reservoir. The channels had a 50 μm × 50 μm squared cross section. A

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Plexiglas holder was fabricated for accommodating the separation chip. Short pipet tips were inserted into each of the four holes on the PMMA chip for providing solution contact between the channel on the chip and corresponding reservoirs on the chip holder.

The homemade dual high-voltage power supply had an adjustable voltage range between −3500 and +3500 V. An Agilent 33120A function generator (Agilent, Palo Alto, CA, USA) was used for generating the sinusoidal ac waveform for the conductivity detection. The electronic circuitry of the contactless conductivity detector was placed on the top of the chip.[10] The circuitry was designed in accordance with a previously reported scheme.[10] This scheme allows convenient interface to data acquisition systems (chart recorder or a computer DAQ). The electronic components were purchased from local suppliers. The electronic circuit was placed in a 90 × 44 × 24 mm shielding box (90 g) to protect the electronics from external electric fields. The open side of the box was placed as close as possible to the sensing electrodes (on the chip), so that the box also acts as a shield for the electrodes. To further minimize the noise, the chip (along with the printed electronic board) was secured from possible mechanical vibrations using a homemade holder.

Fabrication of electrodes

The rectangular-shaped electrodes (0.8 mm × 10 mm) were fabricated from two 10 μm thick aluminium-foil strips. The end side of the electrode was widened to 4 mm to facilitate the electrical connection. The electrodes were fixed on the top of the 125 μm thick PMMA cover plate using a common epoxy, at a distance of 570 μm from the end of the microchannel and with a distance of 700 μm between them. The thin copper wires (20 mm long) were attached to the electrodes using a conducing epoxy (Chemtronics, Kennesaw, GA, USA) and were tin-soldered to the detector electronics. The electrodes were placed in an 'anti-parallel' orientation to minimize the stray capacitance between them (Fig. 1E,e) and to enhance the S/N ratio.[14]

Reagents

Histidine (His), 2-(*N*-morpholino)ethanesulfonic acid (MES), ammonium nitrate, ammonium perchlorate, potassium perchlorate, sodium nitrate and sodium chloride were purchased from Sigma. Methylamine solution was purchased from Fluka. The run buffer (mixture of 20 mM MES and 20 mM His, pH 6.1) was prepared by dissolving MES and His in deionized water. Stock solutions of the target analytes (ammonium, methylammonium, potassium, sodium, chloride, nitrate and perchlorate; at 10 mM) were prepared by dissolving the corresponding salts in the run buffer. All chemicals were used without any further purification.

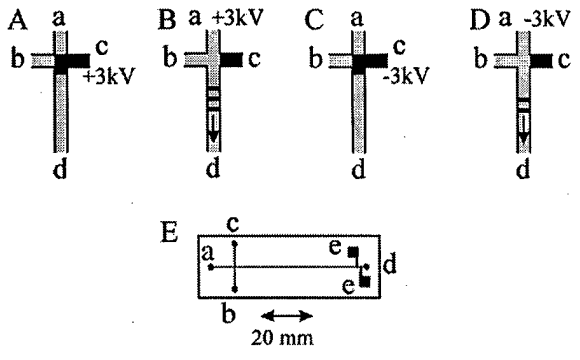

Fig. 1 Schematic of the single-channel microchip assays of explosive-related cations and anions. (A) Injection of cations, (B) separation and detection of cations, (C) injection of anions, (D) separation and detection of anions. (a, b, d) Reservoirs containing run buffer, (c) sample reservoir. Injection field strength +83 V cm^{-1} (A) and −83 V cm^{-1} (C) between reservoir (c) and (d); Separation field strength, +500 V cm^{-1} (B) and −500 V cm^{-1} (D) between reservoirs (a) and (d). (E) Top view of the microchip electrophoretic system with the contactless conductivity detection. Run buffer reservoir (a), unused reservoir (b), sample reservoir (c), outlet reservoir (d), aluminium sensing electrodes (e).

Procedure

Prior to use the channels of the PMMA chip were treated by rinsing with deionized water for 10 min. Reservoirs (a), (b), and (d) (Fig. 1) were filled with the electrophoretic run buffer solution, while reservoir (c) was filled with the sample mixture (target cations and anions dissolved in the run buffer). The following procedure was carried out for measurements of both explosive-related cations and anions. The cations were injected first into the separation channel by applying a field of +83 V cm^{-1} for 3 s between the sample (c) and detection (d) reservoir to ensure loading of sufficient amount of sample (Fig. 1,A). Subsequently, the cation separation was performed by applying +500 V cm^{-1} between the run buffer (a) and detection (d) reservoir (Fig. 1, B). Immediately after detecting the last cation peak of the electrophoregram, a negative field strength of −83 V cm^{-1} was applied for 2 s to the sample (c) and detection (d) reservoir, to ensure injection of the anionic part of the sample (Fig. 1, C). The anion separation was then performed by applying a separation field of −500 V cm^{-1} between the run buffer (a) and detection (d) reservoirs (Fig. 1, D). Some experiments involved measurement of mixtures of cations or anions using steps A, B or C, D, respectively, in Fig. 1. The contactless conductivity detection was accomplished with a frequency of 200 kHz with peak-to-peak amplitude of 5 V.

Safety Considerations. *The high-voltage power supply should be handled with extreme care. The chemicals are irritants and skin and eye contact and accidental inhalation or ingestion should be avoided.*

Results and discussion

The PMMA chip material offers a significant advantage due to its favorably low electroosmotic flow (μ_{EOF} = 1.06 × 10^{-4} cm^2 Vs^{-1}, in our case). Accordingly, the cation and anion separation can be carried out in the cathodic and anodic modes, respectively, using the same run buffer, without adding an electroosmotic-flow reversor. This allows switching between analyses of cations and anions using the same microchannel and run buffer, hence facilitating the rapid detection of explosive-related ions. The absence of an EOF modifier leads also to a lower background conductivity and therefore to an enhanced signal-to-noise ratio.

The new ionic-explosive CE microchip couples the distinct advantages of the contactless conductivity detection scheme with the attractive performance of microfluidic devices. Such performance characteristics compare favorably with those common to chip-based direct-contact conductivity detectors,[17,18] and classical CZE-indirect UV detection.[2] For example, Fig. 2 displays typical electropherograms obtained with the CE-microchip contactless conductivity system for mixtures of pre-explosive inorganic cations [A, a; ammonium (1), potassium (2) and sodium (3)] and inorganic anions [A, b; nitrate (5) and perchlorate (6)] and of post-blast cations [B,a; ammonium (1), methylammonium (4), potassium (2) and sodium (3)] and post-explosive anions [B,b; chloride (7), nitrate

(5) and perchlorate (6)]. The assay has been carried out using separation potentials of +500 V cm^{-1} and –500 V cm^{-1} for cations and anions, respectively (with switching from +500 to –500 V upon completing the cation analysis in connection to a 2 s anion loading (*e.g.* Fig 1; C, D). All four cations and three anions display well-defined and resolved peaks. The half-peak width ranges from 0.6 s (ammonium) to 1.2 s (nitrate). Such a defined response, coupled to the low noise level, offers convenient quantification of these micro- and millimolar levels of studied ions. The total analysis of cations is 25 s, while the analysis of anions is completed within 20 s; accordingly, all seven explosive-related cations and anions can be rapidly measured within a total run time of 50 s. Such short assay times are extremely attractive for providing early and timely detection of inorganic explosive residues. The electrophoretic profiles of Fig. 2 hold great promise for fingerprint identification of a given explosive sample (with the peak ratios offering further confirmation of the sample identity). In view of potential interferences from non-target co-existing ions, it is desired to use the entire electrophoregram for fingerprint identification of the bomb used in each incident.[2]

An effective analysis of pre- and post-explosive residues requires separation of co-existing potassium and ammonium cations. These ions commonly present in explosive samples, have similar mobilities, and hence similar migration times. The addition of 18-crown-6 ether (forming a complex with K^+) has been suggested for facilitating the separation of these explosive-related cations in conventional CE systems.[19] Fig. 3 examines the influence of the crown-ether concentration upon the migration of four explosive-related cations. Increasing the concentration of the 18-crown-6 ether from 0 to 10 mM shifts the migration time of the potassium ion in a nearly linear fashion from 26 s to 38 s (in connection to a separation field of +250 V cm^{-1}). A crown ether concentration of 7.5 mM offers the most favorable separation of the four cations (electropherogram D, right panel). This crown-ether level was thus used for all subsequent work.

Experimental parameters affecting the detection of explosive-related ions were evaluated and optimized. The influence of the separation field strength upon migration time and the conductivity signal is displayed in Fig. 4. As expected, increasing the separation field from +83 V cm^{-1} to +583 V cm^{-1} dramatically decreases the migration time of the cationic ammonium (1), methylammonium (2), potassium (3) and sodium (4) species from 85.0 to 9.5 s, from 102.5 to 11.8 s, from 113.0 to 13.0 s and from 126.5 to 14.8 s, respectively (Fig. 4A). Increasing the separation field from −83 V cm^{-1} to −583 V cm^{-1} dramatically decreases the migration time for the chloride (5), nitrate (6), perchlorate (7) anions from 87.0 to 10.4 s, from 95.0 to 11.5 s and from 103.0 to 12.5 s, respectively (Fig. 4B). Also shown are the resulting electrophoregrams for separation fields +167 (A,a), +333 (A,b) and +500 (A,c) V cm^{-1} for cations and −167 (B,a), −333 (B,b) and −500 (B,c) V cm^{-1} for anions. The shorter migration times observed at higher fields are coupled to substantially sharper peaks. The plate number for the four cations thus increases from 2504 to 3127 (ammonium), from 2877 to 3062 (methylammonium), from 2832 to 3098 (potassium) and from 4542 to 4952 (sodium) over the +83 and +583 V cm^{-1} range. The plate number for the three anions increases from 1165 to 1698 (chloride), from 2002 to 2933 (nitrate) and from 2676 to 3815 (perchlorate). Obviously, no detector zone broadening is expected for placement of the detector on the chip cover. The number of theoretical plates per meter of the present microchip for sodium ion (77,000–84,000 N m^{-1}) compares favorably with those of other conventional (33,000–70,000 N m^{-1})[9] or microchip CE systems (6,450-15,800).[20] The separation voltage field has a negligible effect upon the peak-to-peak background noise level for voltages ranging from +83 V cm^{-1} to +500 V (for cations) and from −83 V cm^{-1} to −500 V cm^{-1} (for anions). Separation field strengths higher than +500 V cm^{-1} resulted in higher

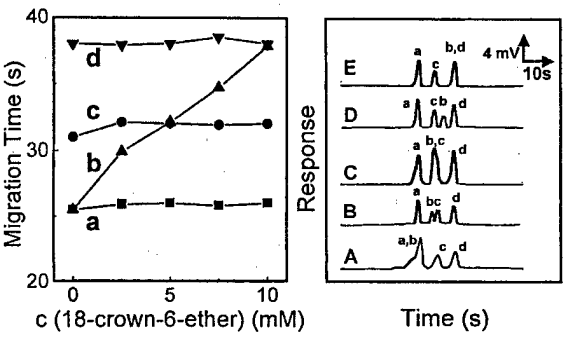

Fig. 3 Effect of 18-crown-6 ether upon the separation of explosive-related cations. Left panel: Influence of the 18-crown-6-ether concentration on the migration times of post-explosive residue cations, ammonium (a), potassium (b), methylammonium (c), sodium (d), sample concentration 350, 350, 400 and 600 μM, respectively. Right panel: The actual electropherograms at different 18-crown-6 ether concentrations: (A) 0 mM, (B) 2.5 mM, (C) 5.0 mM, (D) 7.5 mM, (E) 10.0 mM. Separation field strength, +250 V cm^{-1}; other conditions, as in Fig. 2a.

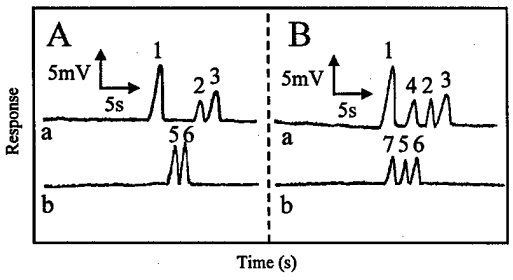

Fig. 2 Electrophoregrams showing the separation of pre-explosive (A) and post-explosive (B) model mixtures of cations (a) and anions (b) using contactless conductivity detection. (A, a): ammonium (1), potassium (2), sodium (3), each at 700 μM. (A,b): nitrate (5), perchlorate (6), sample concentration 1050 μM. (B,a): ammonium (1), methylammonium (4), potassium (2), sodium (3), sample concentration 700 μM. (B,b): chloride (7), nitrate (5), perchlorate (6), sample concentration 700 μM. Conditions a: Separation field strength +500 V cm^{-1}; injection voltage +83 V cm^{-1}, 3 s; frequency 200 kHz; peak-to-peak amplitude 5 V_{p-p}; MES–His buffer (20 mM, pH 6.1) as the run buffer. Conditions b: Separation field strength –500 V cm^{-1}; injection voltage −83 V cm^{-1}, 2 s, other conditions, as in Fig. 2a.

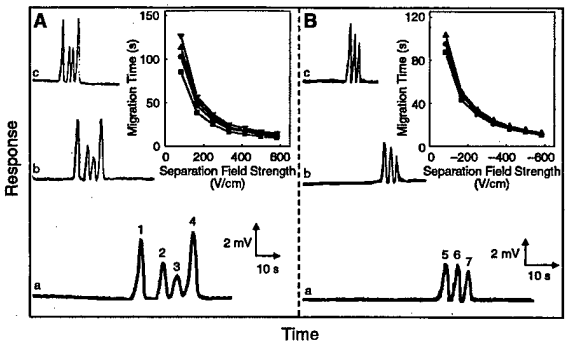

Fig. 4 A: Influence of separation field strength upon the response of 350 μM ammonium (1), 350 μM methylammonium (2), 400 μM potassium (3) and 600 μM sodium (4) at separation field strength (a) +167 V cm^{-1}; (b) +333 V cm^{-1}; (c) +500 V cm^{-1}. Also shown (as inset) the influence of separation voltage upon resulting times in +83 V cm^{-1} steps. Other conditions as in Fig. 2a. B: Influence of separation field strength upon the response of 800 μM chloride (5), 800 μM nitrate (6), 600 μM perchlorate (7) at separation field strength (a) −167 V cm^{-1}; (b) −333 V cm^{-1}; (c) −500 V cm^{-1}. Also shown (as inset) the influence of separation voltage upon resulting times in −83 V cm^{-1} steps. Other conditions, as in Fig. 2b.

background and noise levels (attributed to Joule heating effects). Yet, a flat baseline is observed even at +583 V cm^{-1} and −583 V cm^{-1}, respectively, reflecting the effective isolation of the detector.

The response of the contactless conductivity detector is strongly dependent upon the frequency and amplitude of the applied voltage. The influence of the applied ac voltage frequency is shown in Fig. 5. The curves were recorded pointwise over the 50 kHz to 1000 kHz range for a sample containing ammonium (a), methylammonium (b) and sodium (c) ions. All three explosive-related cations exhibit a similar trend. The response increases slowly between 50 and 400 kHz, then more rapidly (reaching the maximum at 700 kHz) and decreases sharply at higher frequencies. Unstable and distorted response peaks were observed over the 400–900 kHz range (note the larger error bars). A negative signal was also observed in front of each cation peak at frequencies higher than 400 kHz. For example, the inset of Fig. 5 displays the corresponding electrophoregrams at frequencies 200 kHz (A) and 700 kHz (B). It is clear that low frequencies lead to more favorable signal-to-noise characteristics and a well-defined response. The exact reason for the peak distortion and fluctuations observed at high frequencies is not fully understood in view of the complex nature of the registered high-frequency impedance signal. It can be attributed to the complicated equivalence circuit of the detection cell[10,11]and/or to changes in the dielectric properties of the solvent.[12] All subsequent work employed a frequency of 200 kHz which offered the most favorable response characteristics.

The influence of the peak-to-peak amplitude of ac voltage ($V_{p\text{-}p}$) upon the ammonium and methylammonium signals was studied in the range 0–10 $V_{p\text{-}p}$ (Fig. 6). The response increases linearly over the range 0–5 $V_{p\text{-}p}$ and starts to level off thereafter. Such amplitude change results also in a nearly linear increase of the noise level and in a reduced baseline stability. The most favorable signal-to-backround characteristics were obtained using a voltage of 5 $V_{p\text{-}p}$.

The contactless conductivity microchip detector displays a well-defined concentration dependence for both cationic and anionic explosive-related species. A linear range of almost three orders of magnitude (from 20 μM to 7 mM; correlation coefficients, >0.998) was obtained for ammonium and nitrate. Limits of detection of 3.2, 5.8, 6.2, 5.6, 8.7, 7.2 and 6.2 μM were estimated for ammonium, methylammonium, potassium, sodium, chloride, nitrate and perchlorate, respectively [based on the signal-to-noise characteristics (S/N = 3) of the response of a mixture containing 20 μM of these ions; conditions, as in Fig. 2]. These detection limits favorably meet the need of real-life explosive analysis.[2] Further lowering of the detection limits can be expected upon lowering the run buffer concentration.[10] The new microchip CE-contactless conductivity system is characterized also with good reproducibility and stability (associated with the absence of unwanted reactions). For example, series of 10 repetitive injections of a mixture containing 350 μM ammonium, methylammonium, potassium, sodium, chloride, nitrate and perchlorate ions yielded standard deviations of 4.8%, 4.2%, 4.5%, 4.4%, 5.0%, 5.8% and 5.4%, respectively. The stability of the response is attributed in part to the absence of unwanted surface-passivation reaction associated with the contactless configuration.

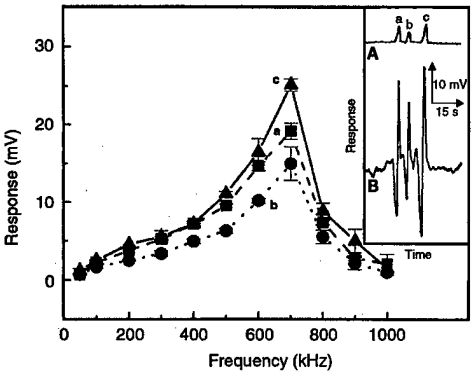

Fig. 5 Effect of the ac voltage frequency upon the response. Sample containing 350 μM ammonium (a), 500 μM methylammonium (b) and 700 μM sodium (c) (peak height). Also shown (as inset) the corresponding electrophoregrams at operation frequency of detector 200 kHz (A) and 700 kHz (B). Separation field strength +250 V cm^{-1}, other conditions, as in Fig. 2a.

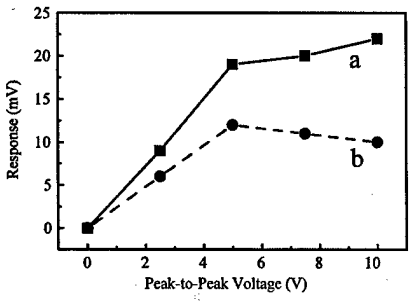

Fig. 6 Influence of the applied peak-to-peak amplitude upon the detector output. Sample contained 350 μM ammonium (a) and 500 μM methylammonium (b). Other conditions, as in Fig. 5.

Conclusions

We have demonstrated a chip-based CE-conductivity microsystem for the rapid separation and detection of explosive-related ions. The new microfluidic device offers significant advantages in term of speed, cost and sample size. Such rapid identification of inorganic explosive residues should have a major impact upon the protection of first responders, and upon the prevention of terrorist activity. On-going efforts in these laboratories are aimed at developing a multi-channel 'counter-terrorism' microchip for providing early and timely detection of different classes of explosives and chemical warfare agents. We are also developing a continuous sampling capability in connection to 'world-to-chip' interface. Further improvements towards mass-production and reproducible electrodes placement can be achieved by using common microfabrication technologies (*e.g.* lithography or screen-printing).

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